REMARKS

This Reply is responsive to the Office Action dated January 6, 2003. Entry of the amendments and remarks submitted herein and reconsideration of the claimed subject matter pursuant to 37 CFR §1.112 is respectfully requested.

At the outset, claims 16 and 17 were amended above to indicate that the claimed DNAs encode proteins having Rho-GEF activity. Support for these amendments may be found in the specification at the very least at page 12, lines 3-4, and at the paragraph bridging pages 22-23. Claims 26 and 28 were amended to refer to nucleic acids encoding at least ninety consecutive amino acids of SEQ ID NO: 2. Support for these amendments may be found at the very least at page 13, lines 2-13. Claim 34 was amended to indicate that the claimed nucleic acid could also be isolated by RT-PCR amplification using the recited primers. Support for this amendment may be found at the very least at page 17, lines 2-12, and page 30, lines 1-7. No prohibited new matter has been added by way of these amendments.

Turning now to the Office Action, Applicants acknowledge with appreciation the indication that claim 25 has been found to be allowable. In addition, it appears that claim 15 should also be declared allowable, since it was not included in any of the rejections set forth in the Office Action. Confirmation of the allowability of claim 15 is respectfully requested.

On page 3 of the Office Action, the Examiner has accorded claim 34 with a priority date of May 26, 2000, *i.e.*, the filing date of the instant application, because SEQ ID Nos. 21 and 22 as recited in claim 34 were allegedly not disclosed in priority

application PCT/JP98/05348. In addition, the Examiner has rejected claim 34 as containing new matter under 35 U.S.C. §112, first paragraph, because the present application was filed as a §371 national stage application of PCT/JP98/05348 and not a CIP.

Applicants respectfully submit that the disclosure of the present application is identical to that of PCT/JP98/05348, as established by the translator's verification filed May 26, 2000. Accordingly, SEQ ID Nos. 21 and 22 were disclosed in PCT/JP98/05348, and the priority date of claim 34 is at the very least the filing date of the PCT priority application, or Nov. 27, 1998. This is further evidenced by the 17 pages of sequence listing from the PCT application filed on May 26, 2000, which contains 22 sequences including the instant SEQ ID Nos 21 and 22.

Further, while the specific example in the specification exemplifying the use of primers having the sequences SEQ ID Nos. 21 and 22 (Example 3) was not included in Japanese priority application 1997-342060 filed Nov. 27, 1997, as evidenced by the translation submitted March 19, 2002, Applicants nevertheless submit that the Japanese priority application fully discloses the limitations of claim 34. For instance, as described on page 15 of priority application JP 1997-342060, beginning at line 13:

> The aforementioned DNA according to the present invention can be synthesized on the basis of one of the base sequences determined by the invention as indicated in the Embodiments below. The DNA can also be obtained from chromosomal DNA by hybridization or *PCR using a probe or an oligonucleotide primer prepared based on the base sequence. Alternatively, the DNA can be obtained by carrying out RT-PCR using cartilage mRNA*, and screening the cartilage or other cDNA library using a polynucleotide having a base sequence encoding all or part of CDEP [sic] as a probe. (With emphasis.)

Further, the sequences identified by SEQ ID Nos. 21 and 22 are included in the nucleotide sequence of CDEP as shown in the sequence listing attached to the translation of priority application JP 1997-342060 (at positions 1733-1752 and 2482-2501, respectively).

Thus, priority application JP 1997-342060 discloses the CDEP cDNA sequence, which includes SEQ ID Nos. 21 and 22, and further discloses that a CDEP DNA can be isolated by PCR or RT-PCR from DNA or RNA, respectively, using primers based on the disclosed nucleotide sequence of CDEP. Accordingly, claim 34, which is directed to an isolated nucleic acid molecule obtained by PCR or RT-PCR amplification using primers having the sequences of SEQ ID Nos. 21 and 22, is fully supported by priority application JP 1997-342060 and should be accorded a priority date of Nov. 27, 1997, *i.e.*, the filing date of the JP priority application.

In view of the comments above, Applicants respectfully request reconsideration and withdrawal of the new matter rejection of claim 34. Further, Applicants respectfully request that claim 34 be accorded a priority date of Nov. 27, 1997.

Beginning on page 4 of the Office Action, claims 16-17, 27-28 and 32 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking written description in the specification. The Office Action also indicates that by typographic error, claim 26 was inadvertently not recited in the previous Office Action, but that it is clear that claim 26 would also be included in the rejection. Applicants respectfully traverse the rejection in view of the amended claims above.

The Examiner's main issue with claims 27 and 28 appears to be the recitation of the word "complementary," as the Examiner believes that a "complement could be a partial or full complement," and that "a partial complement could share with SEQ ID NO: 1 or fragments of at least 180 base pairs of SEQ ID NO: 1 only a few nucleotides" (Office Action, Jan. 6, 2003, page 5). The Examiner's main issue with claim 26 appears to be that a nucleic acid comprising a fragment of at least 180 base pairs of the claimed sequence would encompass any nucleic acid sequence of any length that shares at least 180 base pairs in common with the claimed sequence. The Examiner's main issue with claims 16 and 17 appears to be the lack of a functional limitation in these claims that would provide a common attribute that identifies a variant falling within the scope of the claims, seeing as expression in differentiated chondrocytes versus dedifferientiated chondrocytes is not a specific function of a protein (see Office Action, Jan. 6, 2003, paragraph bridging pages 6-7 and page 7, first full paragraph).

Before addressing the issues raised in the Office Action, Applicants would like to clarify the limitations of claims 26 and 28. Applicants apologize to the Examiner for any statements made in the previous Reply that may have confused the meaning and scope of claims 26 and 28. Claim 26 as amended is directed to an isolated nucleic acid molecule comprising a fragment that encodes at least ninety consecutive amino acids of SEQ ID NO: 2. Claim 28 as amended is directed to an isolated nucleic acid molecule comprising a fragment that is complementary to a nucleic acid which encodes at least ninety consecutive amino acids of SEQ ID NO: 2. Thus, the claimed nucleic acids would encompass 270 base pairs of sequence encoding at least ninety consecutive amino acids

of the protein defined in SEQ ID NO: 2 (rather than 180 base pairs as argued in the previous Reply filed March 19, 2002, at page 18).

Turning to the Examiner's first basis for the rejection, Applicants respectfully disagree with the Examiner's position that a "complement" would be understood by one of skill in the art to include sequences having only a few complementary nucleotides. If this were the case, every known nucleic acid sequence would be a "complement" of every other, since there are only four main nucleotide species that form the genetic code. Rather, a complementary nucleic acid would be understood by one of skill in the art to be fully complementary unless otherwise stated. In any case, Applicants believe that this is clear in view of the disclosure at page 15, lines 13-14, which defines a double-stranded nucleic acid as including two "complementary" strands.

Claim 27 is directed to an isolated nucleic acid molecule consisting of a DNA sequence complementary to the sequence of SEQ ID NO: 1. Given the well known meaning of "complementary" as discussed above, and given the "consisting of" limitation in the claims, claim 27 encompasses only nucleic acid molecules that are fully complementary to SEQ ID NO: 1.

Claim 28 is directed to an isolated nucleic acid molecule comprising a fragment that is complementary to a nucleic acid which encodes at least ninety consecutive amino acids of SEQ ID NO: 2. Given the well known meaning of "complementary" as discussed above, claim 28 encompasses only nucleic acid molecules comprising fragments that are fully complementary to a nucleic acid which encodes at least ninety

consecutive amino acids of SEQ ID NO: 2. Thus, reconsideration of this ground for the rejection is respectfully requested.

Turning to the second basis for the rejection, the Examiner's main issue with claim 26 appears to be that a nucleic acid comprising a fragment of at least 180 base pairs of the claimed sequence would encompass any nucleic acid sequence of any length that shares at least 180 base pairs in common with the claimed sequence. This issue would also be applicable to claim 28 notwithstanding the well-known meaning of the term "complementary." However, this ground for the rejection appears to be moot in view of the amended claims 26 and 28, which are directed to nucleic acid molecules encompassing fragments which encode at least ninety *consecutive* amino acids of SEQ ID NO: 2, and complements thereof.

Turning to the third basis for the rejection, the Examiner's main issue with claims 16 and 17 appears to be the lack of a functional limitation in these claims that would provide a common attribute that identifies a variant falling within the scope of the claims. Apparently, the indication that a variant falling within the scope of the claims is expressed in differentiated chondrocytes versus dedifferientiated chondrocytes is not sufficient, since this attribute is not a specific function of a protein and it is an attribute that would be shared by several other proteins that are specific for differentiated chondrocytes. Without necessarily agreeing with this ground for the rejection, Applicants note that claims 16 and 17 have been amended above to indicate that variants falling within the scope of the claims have Rho-GEF activity. Therefore, this ground for the rejection is now moot.

Reconsideration and withdrawal of the rejection of claims 16-17, 26-28 and 32 in view of the amendments and remarks submitted above are respectfully requested.

Claims 16 and 17 were also rejected under 35 U.S.C. §112, first paragraph, for scope of enablement. Essentially, it is the Examiner's position that, although methods for replacing, deleting or inserting particular amino acids are well known in the art, one of skill in the art would not know how to screen for the claimed nucleic acids without a known function. This rejection also appears to be rendered moot by the amendments to claims 16 and 17 above, which added the functional limitation that the claimed variants have Rho-GEF activity. The knowledge of how to screen for this activity was well known to those of skill in the art at the time of the invention and is supported in the instant specification. See, for example, the paragraph bridging pages 22 and 23. Reconsideration and withdrawal of the rejection is respectfully requested.

Claims 27-28 and 32 were rejected for lack of enablement under 35 U.S.C. §112, first paragraph, because the claimed "complementary" sequences could allegedly share only a few nucleotides in common with SEQ ID NO: 1. At the outset, Applicants note that, while claim 27 is directed to nucleic acids that are complementary to the sequence of SEQ ID NO: 1, claim 28 is directed to nucleic acids that are complementary to a nucleic acid encoding at least ninety amino acids of SEQ ID NO: 2. Because of the triplet code, a claim that encompasses nucleic acids that encode a recited protein sequence is broader than a claim directed solely to a specific nucleic acid sequence. That being said, the claims do not encompass sequences that share "only a few" nucleotides in common with

SEQ ID NO: 1 or a few nucleotides in common with nucleic acids encoding ninety amino acids of SEQ ID NO: 2, as alleged in the Office Action (see page 9 of the Office Action).

Indeed, as discussed above with reference to the rejection based on lack of written description, Applicants respectfully disagree with the Examiner's position that a "complement" would be understood by one of skill in the art to include sequences having only a few complementary nucleotides. If this were the case, every known nucleic acid sequence would be a "complement" of every other, since there are only four main nucleotide species that form the genetic code. Rather, a complementary nucleic acid would be understood by one of skill in the art to be fully complementary unless otherwise stated. In any case, Applicants believe that this is clear in view of the disclosure at page 15, lines 13-14, which defines a double-stranded nucleic acid as including two "complementary" strands.

Furthermore, claim 28 has been amended above to indicate that the claimed nucleic acids comprise fragments that are complementary to a nucleic acids encoding at least ninety consecutive amino acids of SEQ ID NO: 2. In view of the well-known definition of complementary and the amendment to claim 28, reconsideration and withdrawal of the rejection of claims 27 and 28 under §112, first paragraph for lack of enablement is respectfully requested.

Claims 17 and 32 were rejected for scope of enablement under 35 U.S.C. §112, first paragraph, because the claims do not reasonably provide enablement for a gene comprising a DNA fragment of SEQ ID NO: 1, or a DNA which hybridizes under stringent conditions to a fragment of SEQ ID NO: 1. The Examiner's main issue in this

rejection appears to be that the claims read on a gene or genomic DNA that contains 5' and 3' regulatory regions and untranslated regions that are not described in the specification (see page 10 of Office Action dated Jan. 6, 2003). Applicants respectfully traverse the rejection.

The Examiner is correct to point out that the specification does not describe a genomic sequence encoding a CDEP protein. However, it would not require undue experimentation to identify such sequences based on the disclosure combined with the level of skill in the art. For instance, at page 18, lines 12-19, it is disclosed that genes encoding a CDEP protein may be isolated following southern blot hybridization using a probe based on the disclosed amino acid and nucleotide sequences. In addition, at page 17, lines 5-7, it is disclosed that a CDEP gene can be obtained from chromosomal DNA by PCR or hybridization using oligonucleotide primers or a probe based on the disclosed nucleotide sequence.

Further, according to Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual (2d edition) (relevant pages attached hereto), libraries generated from mammalian genomic DNA had been in use since the mid-1970's for cloning mammalian genes (see page 9.2). And according to the teachings on page 9.3, it was well-known at the time this Manual was published that one could use libraries of randomly cleaved DNA to "walk" along the eukaryotic chromosome starting with a single specific probe, in order to isolate segments of DNA in and around target sequences without knowledge of the location of surrounding restriction sites. Thus, it was common practice at the time the present application was filed to isolate a genomic DNA corresponding to a known cDNA

sequence following hybridization of a probe to a genomic library. Moreover, such common techniques need not be described, because the specification need not disclose what is common knowledge in the art.

Seeing as the specification states that CDEP sequences can be isolated from chromosomal DNA following hybridization with a specific probe, and seeing as it was common knowledge since the mid-1970's (over twenty years prior to the making of the present invention) that one can isolate genomic DNA in and around target sequences starting with a single specific probe, identifying and isolating genomic sequences that encompass or hybridize to the disclosed CDEP sequence would not have required undue experimentation at the time the application was filed. Reconsideration and withdrawal of the rejection of claims 17 and 32 for scope of enablement is respectfully requested.

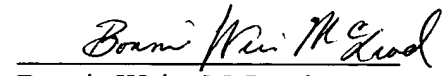
Claim 34 was rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Koyano et al. (1997) (BBRC, 241(2): 369-75). This article was published on December 18, 1997, as shown by the PubMed abstract submitted with the previous Reply filed March 19, 2002. Therefore, this article is not prior art to claim 34, which is entitled to a foreign priority date of November 27, 1997, as discussed above. Reconsideration and withdrawal of the rejection is respectfully requested.

This reply is fully responsive to the Office Action dated January 6, 2003. Therefore, a Notice of Allowance is next in order and is respectfully requested.

Except for issue fees payable under 37 CFR §1.18, the commissioner is hereby authorized by this paper to charge any additional fees during the pendency of this application including fees due under 37 CFR §1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 CFR §1.136(a)(3).

If the Examiner has any further questions relating to this Reply or to the application in general, she is respectfully requested to contact the undersigned by telephone so that allowance of the present application may be expedited.

Respectfully submitted,



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Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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Cold Spring Harbor Laboratory Press
1989

If current estimates are correct, we shall move in the short span of 50 years from the discovery of the structure of DNA to the elucidation of the complete sequence of the human genome. Although this rapid rate of progress has been catalyzed by many theoretical and technical advances, none has been so informative as the ability to construct libraries of eukaryotic genomic DNA. Originally devised as banks from which individual bacterial genes could be isolated and analyzed (Clarke and Carbon 1976), genomic DNA libraries are now increasingly viewed as matrices that can be assembled into maps of the genome from which they were originally derived.

The feasibility of using libraries in this way was established in the late 1970s, when Maniatis et al. (1978) devised a strategy to generate and clone large numbers of random fragments of mammalian genomic DNA. Before then, genomic libraries consisted of fragments of DNA obtained by complete digestion of genomic DNA with a restriction enzyme such as *Eco*RI. The earliest libraries of mammalian genomic DNA contained only subsets of this large population of fragments because it was necessary to enrich for the sequences of interest due to the comparative inefficiency of packaging of bacteriophage λ DNA. For example, Tilghman et al. (1977) constructed a library from fragments of mouse DNA that had been enriched approximately tenfold for β-globin sequences by preparative gel electrophoresis and reversed-phase chromatography (Hardies and Wells 1976). The improvement of in vitro packaging systems (Hohn and Murray 1977; Sternberg et al. 1977) and the development of in situ hybridization techniques (Benton and Davis 1977) soon allowed much larger libraries to be constructed from unfractionated populations of restriction fragments (Maniatis et al. 1978; Smithies et al. 1978), thus eliminating the need to enrich the starting DNA for the sequences of interest. The inherent limitations of genomic DNA libraries constructed from complete digests of genomic DNA with a restriction enzyme such as *Eco*RI then quickly became apparent. These limitations include the following:

- Because of their large size, libraries of complete digests are laborious both to create and to screen. The average distance between *Eco*RI sites in the mammalian genome is approximately 4 kb (Botchan et al. 1974), and complete digests therefore contain approximately 10^6 different fragments of DNA. Consequently, a library constructed from these fragments needs to contain almost 10^7 independent recombinants to have a reasonable chance of including a desired sequence.
- If the sequence of interest contains one or more recognition sites for the particular restriction enzyme, it will be cloned in two or more nonoverlapping recombinants. In libraries of complete restriction digests, there is no way to "walk" from one recombinant clone to another that contains the neighboring restriction fragment in the original genomic DNA.
- Because of the quasi-random distribution of restriction sites in mammalian DNA, the sequence of interest may by chance be located on a fragment of genomic DNA that is too large or too small for the vector to accept.

The solution to these problems was to clone large pieces of DNA generated

either by mechanical shearing (Wensink et al. 1974; Clarke and Carbon 1976) or by partial digestion with restriction enzymes that cleave the target DNA frequently (Maniatis et al. 1978). Unfortunately, the efficiency of cloning sheared DNA was not sufficient to obtain representative libraries. Thus, virtually all of the libraries used to isolate single-copy genes were produced by partial digestion of genomic DNA with restriction enzymes. These DNA fragments were fractionated by density gradient centrifugation or preparative gel electrophoresis to select those whose size was suitable for insertion into the vector (Maniatis et al. 1978). This method, which is used in an essentially unchanged form today, helps to ensure that there is no systematic exclusion of sequences from the cloned library merely because of an unfortunate distribution of restriction sites. The following advantages are gained by constructing libraries of randomly cleaved DNA:

- *The ability to "walk" along the eukaryotic chromosome in a way that is impossible with a library of nonoverlapping restriction fragments.* Random cleavage of genomic DNA generates collections of overlapping fragments from each chromosome. Thus, a library of these fragments should consist of 46 independent collections of overlapping clones. If the library contains no gaps, the clones forming an individual series will correspond to the entire DNA sequence of an individual chromosome. To walk along a chromosome, a segment of nonrepetitive DNA derived from one end of a given recombinant is used as a probe to identify overlapping clones that contain the adjacent sequence. This process can be repeated until clones have been identified that cover the entire region of interest.
- *The ability to isolate segments of DNA without knowledge of the location of restriction sites in and around the target sequences.*
- *A reduction in the size of the library.* Because the DNA fragments selected for cloning are large, the number of recombinants required to generate a mammalian genomic DNA library is significantly reduced.

Note: The exact probability of having any given DNA sequence in the library can be calculated from the equation

$$N = \frac{\ln(1-P)}{\ln(1-f)}$$

where P is the desired probability, f is the fractional proportion of the genome in a single recombinant, and N is the necessary number of recombinants (Clarke and Carbon 1976). For example, to achieve a 99% probability ($P = 0.99$) of having a given DNA sequence represented in a library of 17-kb fragments of a mammalian genome (3×10^9 bp)

$$N = \frac{\ln(1-0.99)}{\ln\left(1-\left[\frac{1.7\times10^4}{3\times10^9}\right]\right)} = 8.1\times10^5$$